## THE EFFECTS OF INCREASING THE IODINE CON-TENT OF THE TOMATO PLANT ON RESPIRATION AND ENZYMATIC ACTIVITY<sup>1</sup>

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## I. INTRODUCTION

Ever since its discovery in kelp by Courtois in 1811, iodine has been the subject of much biological research, and a truly 'An investigation carried out in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

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enormous literature has developed. The greater number of studies deals with the relation of iodine to animal metabolism, especially to the activity of the thyroid gland. Even before its discovery in the thyroid glands by Baumann ('95), its connection with goitre was suspected, and Fourcault ('51 a, b), Thenard ('51), Chatin ('51-'53), Grange ('52), Casaseca ('53) and others had published their results on the relation of the iodine content of food, water, and air to the prevalence of human goitre and cretinism. According to Muller (Lindley's 'The Vegetable Kingdom,' p. 353) iodine had been found in a cress of unknown origin. This fact was investigated and verified by Chatin ('50 a, b, '66), who may be considered the first to determine positively iodine in the higher plants. Since that time many questions have been raised concerning possible functions of iodine in the plant, but studies attacking these problems have been of little significance. The pertinent literature is so extensive that a satisfactory review can not be attempted here.

The unsatisfactory results of iodine research in plant physiology are perhaps due primarily to the lack of a successful method of growing plants free from iodine, and the absolute necessity of this element to growth is therefore almost impossible to demonstrate. Iodine is omnipresent in the air, according to Chatin ('51-'66), Gautier ('99, '20), and Wagner ('29), and also in water and soil, at least in minute traces. Even if the external experimental habitat were freed from iodine, the seed itself might pass on from generation to generation a biological sufficiency of this element. The present paper reports the results of a study on tomato plants grown in water cultures to which various amounts of iodine as potassium iodide were added. The effects of the increasing amounts of iodine on growth, toxic symptoms, respiration, and enzymatic activity were observed.

Many workers have found that plants absorb iodine more or

less proportionally to the amount present in the soil or culture solution. Stoklasa ('24) grew sugar beets in pots containing 12 kilograms of soil to which .02 gram iodine was added as potassium iodide, and found that in the air-dry tissue the con-

trols contained .32 milligram iodine per kilogram of leaves, and .15 milligram per kilogram of roots, while the plants grown in the iodized pots contained .90 milligram iodine per kilogram of leaves and .60 milligram per kilogram of roots. Other researchers also have found an increase in iodine content on addition of this substance to the substrate, as Stoklasa ('26, '29, '30), Scharrer and Schwaibold ('27), Scharrer and Strobel ('27), Orr, Kelly, and Stuart ('28), Hiltner ('28). The field studies of Wrangell ('27) did not show any increase in iodine with iodine manuring, but as was later pointed out by Orr, Kelly, and Stuart ('28) this may have been due to some abnormal soil condition which made the iodine unavailable. It should be noted also that Klein ('27) in his critical review concluded that the iodine content of plants was held between narrow limits and that it was not possible to vary this significantly. In spite of these dissenting voices, it seems reasonable to conclude that the amount of iodine in plant tissues is dependent to a greater or less degree on the amount available in the substrate. Owing to the fact that all of the various enzymes discussed below were studied from a single lot of plants in order that the conditions of growth would be identical, sufficient tissue was not available for iodine analysis. The maximum possible number of plants was grown in the time permitted, and the tissue proportioned to the different phases of the work. Since the literature gives sufficient evidence concerning the absorption of iodine, all of the tissue available was reserved for the enzyme studies. The results reported below indicate definite physiological effects varying with the different concentrations of potassium iodide in the nutrient solution. This is in itself a proof that increasing amounts of iodide were absorbed. Kostytschew ('26) summarizes the situation as follows: "Es ist festgestellt, dass fermentative Oxydations- und Gärungsvorgänge durch Alkaloide und andere Reizstoffe ausserhalb der Zelle nicht gesteigert werden können. Nun ist auch eine Fermentbildung ausserhalb des lebenden Plasmas bisher nicht bekannt."

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## II. GENERAL METHODS

"Bonny Best" tomato seed were planted February 27, 1932, in flats of greenhouse soil, and the seedlings were transplanted to the water culture jars on March 25. It is, of course, possible that during this month of contact with soil, sufficient iodine was absorbed to mask the effect of the later addition of small amounts to the water cultures. However, the purpose of the work was not to study the necessity of iodine in itself, nor to determine its optimum concentration for growth, but to observe the physiological effects of increasing the iodine content of the plant. Hence the possibility of the absorption of some iodine during this stage was disregarded, since all seedlings were germinated under identical conditions, in the same greenhouse flat.

The nutrient medium used was Shive's ('17) three-salt solution, which he found best for buckwheat tops. Its composition was as follows:

> $\rm KH_2PO_4$ .0144 M Ca(NO<sub>3</sub>)<sub>2</sub> .0052 M  $MgSO_4$ .0200 M

One part per million of manganese as manganous sulphate, boron as sodium borate, and iron as ferrous sulphate were used in all cultures. Potassium iodide was added to give 1, 5, 10, and 20 parts per million of iodine. A control series contained no potassium iodide.

The cultures were grown in one-quart glazed earthenware jars. These were allowed to stand filled with 2N sodium hydroxide for two weeks and with 2N sulphuric acid for two weeks, so that all easily soluble substances would be dissolved from the surface. Sheets of cork six inches square and onehalf inch thick covered the tops of the jars so that no light could reach the roots. Holes one-half inch in diameter were cut in the cork to accommodate the plants. Six plants were placed in each jar. During the first week the solutions were renewed twice, during the second week three times, and daily from then on.

## III. EXPERIMENTAL RESULTS

## A. GROWTH

The earliest worker dealing with the effect of iodine on the growth of plants was Suzuki ('02b). He grew *Pisum* in pots containing 2300 grams of air-dry soil plus .001 grain potassium iodide added six times during the growth period. The iodized soil was reported as causing the weight of fresh fruits to increase from 60.5 to 72.4 grams, the air-dry seed to increase from 23.2 to 26.3 grams, and the air-dry straw to increase from 10.7 to 15.5 grams. Since only one pot each was used for the control and experimental plants, and each pot contained only five plants, it is not possible to draw any positive conclusions from the experiment. The following year Susuki and Aso ('03) reported a stimulation of radish and oat seedlings in pot culture by the addition of potassium iodide. Here again the number of plants was too small to lend significance to the results.

Mazé ('15), noticing that corn grew well in sterile spring water but imperfectly or not at all in distilled water, set out to find the essential elements of the nutrition of this plant. The elements ordinarily present in nutrient solution did not give good growth. The addition of .004 grams of potassium iodide per liter showed a beneficial effect. Budington ('19) observed the growth of onion root tips in 20 cc. of Pfeffer's nutrient solution to which .25 to 1 grain of desiccated thyroid gland was added. Only harmful effects on growth were noted, although when the same amounts of iodine were added as potassium iodide the growth was normal, from which it appears that the thyroid was harmful for some reason other than its iodine content. Stoklasa ('24) found in pot studies that the addition of .02 gram of iodine as potassium iodide to 12 kilograms of soil produced a better development of sugar beets, especially of the leaves. In field studies 1.72 kilograms of iodine as potassium iodide per hectare gave a better growth of sugar beets and caused more and better seeds to develop during the second year. Stoklasa ('26) also found that .009-.015 gram of iodine as

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potassium iodide per 3200 cc. of nutrient solution increased the growth of Hemerocallis fulva almost 100 per cent. Potassium iodide at the rate of .021 gram of iodine per 12 kilograms of earth increased the growth of the leaves and roots of the sugar beets, confirming some earlier observations. Dafert and Brichta ('26), in comparing the value of synthetic nitrate with Chile saltpeter, found that iodine in an amount equal to that in the natural product had no effect on barley, mustard, and turnips. Brenchley ('24) found little or no beneficial effect of an iodine-potassium-iodide mixture on barley or mustard, but suggested that other plants might react differently. Wrangell ('27), using .3-1.5 kilograms of iodine as potassium iodine per hectare, grew a variety of agricultural plants, with no apparent effect on growth. Haas and Reed ('27) found that young orange trees did not thrive after 18-24 months unless the nutrient solution contained .2 part per million of I, Al, Ti, Br, Sr, Si, Mn, B, NH<sub>4</sub>. It does not follow that each of these elements is necessary.

Scharrer and Schwartz ('27) found that small amounts of various inorganic iodine compounds slightly stimulated the multiplication of yeast, but did not increase the maximum yield. This work was followed by that of Greaves, Zobell, and Greaves ('28), who obtained poor growth on iodine-free nutrient solution and vigorous growth with 10 parts per million of iodine as potassium iodide. These authors held iodine to be an essential nutrient for yeast. Engles ('28), through field studies on sugar beets and turnips, concluded that there was no stimulative effect from iodine added as potassium iodide. Stoklasa ('29) again reported a large increase in the growth of sugar beets from potassium iodide manuring. According to Haas ('30), iodine could be omitted from nutrient solutions for citrus plants with no harmful effect. Cotton ('30) found no stimulation to buckwheat growth in nutrient solutions containing concentrations

of potassium iodide of 1.27 to 12.7 parts per million of iodine. Scharrer and Schropp ('31) observed a slight stimulation at

low concentrations of iodine compounds on wheat in pot studies.

From this incomplete review of the literature it is apparent that no agreement has yet been reached concerning the effect of iodine on the growth of plants.

In the present work, only depressing effects on growth were observed. The average of 25 plants after a growth period of

2 months is represented in fig. 1, and the numerical data appear in tables 1 and 11. From the curves it is seen that both the



Fig. 1. Growth data: A, succulence (% of loss in drying); B, dry weight of roots; C, dry weight of tops; D, fresh weight of

## tops.

fresh and dry weight of the tops were about equally depressed. The dry weight of the roots was less depressed, and then only at the higher concentration. The degree of succulence as shown by the loss of weight on drying is apparently unaffected.

### TABLE I

GROWTH DATA IN ABSOLUTE AMOUNTS. AVERAGE BASED ON 25 PLANTS. VALUES TAKEN ON APRIL 27, 1932, AFTER A GROWTH PERIOD OF 2 MONTHS

Concentration of Iodine	Fresh wt. of tops (gms.)	Dry wt. of tops (gms.)	Dry wt. of roots (gms.)	Loss of wt. of tops in drying (gms.)	% of loss in tops by drying
Control	9.4	.84	.28	8.56	91
1 p.p.m.	8.7	.78	.26	7.92	91
5 p.p.m.	6.8	.59	.28	6.21	91
10 p.p.m.	6.2	.57	.24	5.63	91
20 p.p.m.	5.0	.46	.20	4.54	91

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#### TABLE II

SAME AS TABLE I, THE FIGURES INDICATING THE RELATIVE VALUES, UPON WHICH THE GRAPHS OF FIGURE 1 ARE BASED. THE CONTROL IN EACH CASE IS TAKEN AS 100

Concentration of Iodine	Fresh wt. of tops	Dry wt. of tops	Dry wt. of roots	% of loss in tops by drying
Control	100	100	100	100
1 p.p.m.	92	93	93	100
5 p.p.m.	72	70	100	100
10 p.p.m.	66	68 .	85	100
20 p.p.m.	53	55	71	100

### B. TOXIC SYMPTOMS

The evidence of injury due to toxic concentrations of iodine compounds has been described by Suzuki and Aso ('03) and by Mazé ('19). These authors observed brown spots on the foliage. Cotton ('30), in a paper dealing specifically with the toxic effects of iodine and nickel on buckwheat in water culture, verified these observations. She found the spots to be characteristic in aspect. Generally slightly sunken areas appeared first, often near the margins of the leaves. These became pale brown spots encircled by a darker brown, and the areas enlarged and coalesced. The petioles then turned brown at the base and the leaf dropped. In the present work many hundreds of tomato plants were grown in concentrations of potassium iodide that were obviously toxic to the plant, but no brown spotting of the foliage was observed. At a concentration of 1 part per million of iodine as potassium iodide, there was no visible effect on the health of the plant. At 5 parts per million, only rarely did individual plants appear to be unhealthy. Concentrations of 10 and 20 parts per million usually gave plants unhealthy in appearance. Toxicity was first shown by a decrease in the intensity of the green color. This was followed by a definite chlorosis of the leaves, particularly of the lower. It is true that this chlorotic condition developed first in the tissue between the larger veins and later spread over the entire leaf, but strictly speaking these areas could not be defined as spots.

This difference is probably due to the specific reaction of tomato plants, although it is noted that Mazé worked with corn and Cotton worked with buckwheat, and both obtained a characteristic spotting of the leaves. The leaves of our plants were quickly dropped when the chlorotic condition was reached. No specific injury to the growing tips was noted.

C. ACIDITY OF THE PRESS JUICE

Stoklasa ('26) grew Senecio vulgaris in pot culture, with the addition of 0.05 gram of iodine as potassium iodide to each 9.5 kilograms of soil. After 40 days the pH of the press juice was determined. The iodine-manured plants showed a conspicuous increase in pH over the controls. The same result was obtained with Epilobium hirsutum and with Beta vulgaris. This change in acidity he attributed to the breaking down of organic acids due to the iodine in the plant increasing the enzymatic decomposition of these substances.

Since Stoklasa attributed considerable importance to this decrease in acidity because it would tend to stimulate general enzymatic activity in the tissue, a careful study was made of this feature. The press juice from 10 plants was determined by the quinhydrone electrode for each concentration of iodine in the nutrient solution. No significant difference was observed, and the variation in enzymatic activity of the tomato plants described below must be attributed to some other action than the effect of the iodine on the acidity of the press juice.

## D. RESPIRATION

The effect of iodine and iodine compounds on animal respiration has been investigated by a number of authors. Macht and Hooker ('18) studied the action of iodide, bromide, and nitrate ions on the respiratory center of a small dog. Dog's blood was defibrinated and diluted with Locke's solution in which the usual sodium chloride was replaced by sodium iodide. This preparation was perfused into the carotid artery under such conditions that the profusate was practically isolated in the head region. It was found that the iodide ion, as well as the bromide ion, stimulated the respiratory rate, as shown by the deeper amplitude of the respiration curve. Stimulation of the

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respiratory center is equivalent to an increase in intracellular oxidation only in case its activity parallels the demand by the cells for oxygen.

Cameron and Carmichael ('20) found that continued small doses of desiccated thyroid fed to white rats caused, among other things, a hypertrophy of various organs due to an increased metabolic rate. Sodium iodide fed in amounts equaling the iodine of the thyroid up to 100 times as much did not produce this effect, which indicates that it is not iodine itself but a particular iodine compound which is the effective agent.

Hara ('23) found that injections of elementary iodine into the rat in amounts corresponding to 2 grams in man did not affect the metabolic rate, as shown by the comparative oxygen absorption.

Wickwire, Seager, and Burge ('28) observed the effect of various iodine compounds on the rate of sugar utilization by goldfish. Desiccated thyroid, thyroxin, clacidine, potassium iodide, and sodium iodide, respectively, were added to 100-cc. portions of .1 per cent dextrose in such amounts that each portion contained .55 milligram of iodine. Two goldfish were put in each solution. Air was bubbled through, and after 30 hours the sugar remaining in the solution was determined by the Benedict method. Only the desiccated thyroid had any effect. This substance caused an increase of 50 per cent in the sugar utilization over that of the control. These results agree with those of Cameron and Carmichael ('20) that it is not iodine in itself but a definite compound of iodine that stimulates metabolism. Wilhelmj and Boothby ('30) further verified the stimulatory effects of thyroxin on the basal metabolic rate.

Baker, Bacon, Lundy and Klein ('30) found that a thyroid extract injected intravenously in dogs caused a depression of the respiration rate, followed by an increase. The same result was obtained with extracts of kidney, suprarenal gland, liver, pancreas, duodenum, thymus, prostate, and muscle, and therefore nothing can be inferred concerning the actual effect of

iodine. These authors held that the symptoms were not due to thyroxin nor to di-iodo-tyrosin, since none of these substances showed the same amount of iodine present in the tissue extracts.

The discussion of the relation between the function of the thyroid and metabolic activities, particularly in cases of human goitre, is too extensive to review here.

The influence of iodine and iodine compounds on the rate of plant respiration metabolism has been but little studied, in spite of the fact that the results obtained by the animal biologists suggests that this would be a fertile field for research. Lieben and Lászlo ('25) investigated the effect of various ions on sugar metabolism of oxygenated yeast and found that the iodine ion, in common with several other ions, significantly increased the utilization of sugar although there appeared to be no relation between the quantity of these ions present and their effectiveness.

Stoklasa ('26) found that the roots of iodine-poor sugar beets produced 1463 milligrams CO<sub>2</sub> in a definite period, while iodine-rich roots eliminated 1522 milligrams during the same time. In anaerobic conditions and in a pure hydrogen atmosphere this increase did not occur. Comparable results were obtained with potato tubers. This author also held that iodine as potassium iodide in the nutrient medium of Azotobacter chroococcum stimulated respiration, and thereby increased the amount of nitrogen fixed during the process. Greaves, Zobell and Greaves ('28) investigated the effects of iodine and iodine compounds on the rate of reproduction and carbon-dioxide output of yeast. Commercial yeast in the presence of 1 part per million of iodine as potassium iodide showed an increased growth, but the individual metabolic activity was not affected. Comparable results were obtained with sodium iodide, calcium iodide, and iodine. The metabolic activity was increased, however, by these substances in the

presence of maltose or lactose.

Scharrer and Claus ('30) found that 9-hour cultures of beer yeast eliminated less CO<sub>2</sub> when .5 per cent iodine as sodium

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Fig. 3. Respiration Determination 2.

Explanation of graphs.—In all cases, the value of the control plant is calculated as 100, and the potassium-iodide-treated plants as per cents of the control. The numbers on the ordinate represent the per cent stimulation or depression, the graphical value of the control therefore being zero. The horizontal line divides the stimulation and depression values. The numbers on the abscissa represent the parts per million of iodine as potassium iodide in the nutrient solution. Curve "G" represents the growth of the plants used for the determinations indicated by the remaining curves of the same figure.

iodide was present in the culture medium, but with 2.02 per cent iodine there was a conspicuous increase.

As a part of the present investigation the carbon dioxide output from tomato plants growing in water culture was measured. In preliminary work it was found difficult to obtain thorough absorption of carbon dioxide by drawing air through liquid absorbents unless such a quantity of absorbents was used that accurate titration was impossible. Absorption bulbs so constructed that the air bubbles were broken several times gave complete absorption, but the absorbent was difficult to remove completely after each determination. Since liquid absorbents were either inaccurate or inconvenient, an apparatus involving a dry carbon-dioxide absorbent was designed. This has been previously reported by the author ('32), and is pictured in plate 17. In each case, the nutrient solutions were renewed just before the determinations were started in order to eliminate the possibility of inaccuracy due to the activity of microorganisms. The culture jars, each containing from four to six plants, were placed in the bell-jar compartments of the respirometer at 10 o'clock in the evening, and the room was thoroughly darkened to eliminate photosynthetic activity during the early part of the succeeding morning. (For the details of the apparatus and of the procedure of determination, see Wynd, '32). At 8 or 9 o'clock the next morning, the Fleming-Martin absorption bulbs were disconnected and weighed on the analytical balance. The plants were then weighed and the carbon-dioxide elimination per gram of fresh weight of tops was calculated (tables III-IX). Since the relation between the extent of iodine injury and the increase in respiration is of some interest, detailed descriptions are given below.

### **RESPIRATION DETERMINATION 1**

Control—Plants normal in color and appearance. 1 p.p.m.-Plants indistinguishable from the controls.

5 p.p.m.-Plants showing slight injury; less green than the preceding; the lower leaves dropped.

10 p.p.m.-Indistinguishable from plants in the 5 p.p.m. solution. 20 p.p.m.-Indistinguishable from plants in the 5 p.p.m. solution.

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Examination of table III and fig. 2 shows that the increase in respiration is not closely correlated with the extent of injury. Attention may be called to the fact that the plants growing in 20 p.p.m. of iodine showed even less injury as shown by the growth curves, than those in the two preceding concentrations, although their respiration was conspicuously higher.

### TABLE III

### RESPIRATION DETERMINATION 1, DATA TAKEN APRIL 18, 1932

Iodine concen- tration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO <sub>2</sub> output in 11 hrs. per gm. fresh wt. (gms.)	Relative CO <sub>2</sub> output per gm. fresh wt. (con- trol as 100)
Control	6	8.90	100	.00321	100
1 p.p.m.	6	7.54	85	.00289	99
5 p.p.m.	5	5.10	57	.00637	199
10 p.p.m.	6	4.87	55	.00476	148
20 p.p.m.	6	6.64	74	.00842	262

### **RESPIRATION DETERMINATION 2**

Control—Plants entirely normal in color and appearance.
1 p.p.m.—No visible toxic effect; plants entirely similar to the control.
5 p.p.m.—No visible toxic effect; plants entirely similar to the control.
10 p.p.m.—No visible toxic effect; plants entirely similar to the control.
20 p.p.m.—Plants less green, but decrease in color so slight as to be almost indetectable by the eye; a few of the lower leaves dropped.

Examination of table iv and fig. 3 shows that very little toxic effect was shown in this series. A small decrease in fresh weight occurred at 1 p.p.m., but no additional decrease oc-

### TABLE IV

RESPIRATION DETERMINATION 2, DATA TAKEN APRIL 19, 1932

Iodine concen- tration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO <sub>2</sub> output in 10 hrs. per gm. fresh wt. (gms.)	Relative CO <sub>2</sub> output per gm. fresh wt. (con- trol as 100)
Control	6	10.3	100	.00366	100
1 p.p.m.	6	8.3	81	.00374	102
5 p.p.m.	5	7.4	72	.00388	106
10 p.p.m.	6	7.0	68	.00543	149
20 p.p.m.	6	6.0	58	.00904	247

curred at the higher concentrations. Again there appears to be no striking connection between respiratory activity and degree of injury.

## **RESPIRATION DETERMINATION 3**

Control-Entirely normal plants.

1 p.p.m.—Entirely normal, indistinguishable from the controls. 5 p.p.m.—Entirely normal, indistinguishable from the controls.

10 p.p.m.-Lower leaves slightly yellowish. 20 p.p.m.-Entire foliage very slightly chlorotic.



Fig. 4. Respiration Determination 3.

In this series, the 1 p.p.m. plants, while indistinguishable from the controls in appearance, weighed a little more. The data are given in table v and fig. 4. There is obviously no injury

### TABLE V

### RESPIRATION DETERMINATION 3, DATA TAKEN APRIL 20, 1932

Iodine concen- tration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO <sub>2</sub> output in 10 hrs. per gm. fresh wt. (gms.)	Relative CO <sub>2</sub> output per gm. fresh wt. (con- trol as 100)
Control	6	9.5	100	.00569	100
1 p.p.m.	6	9.9	104	.00455	89
5 p.p.m.	6	6.4	67	.00552	97
10 p.p.m.	6	6.7	71	.00924	164
20 p.p.m.	5	6.1	63	.01548	270

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here, but again the respiration at this point is decreased. At higher concentrations, the increase of respiration was not proportional to the degree of visible injury nor to the decrease in weight. This is clearly shown in fig. 4.

## **RESPIRATION DETERMINATION 4**

Control-Plants entirely normal.

1 p.p.m.-Plants entirely normal, similar to the controls.

5 p.p.m.-Lower leaves slightly yellowish.

10 p.p.m.-Plants appearing identical to those in 5 p.p.m.

20 p.p.m.-Whole plant slightly less green than controls; lower leaves dropped.



Fig. 5. Respiration Determination 4.

### TABLE VI

## RESPIRATION DETERMINATION 4, DATA TAKEN APRIL 21, 1932

Iodine concen- tration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO <sub>2</sub> output in 10 hrs. per gm. fresh wt. (gms.)	Relative CO <sub>2</sub> output per gm. fresh wt. (con- trol as 100)
Control	6	9.2	100	.00349	100
1 p.p.m.	6	7.4	79	.00278	80
5 p.p.m.	6	6.4	68	.00300	86
10 p.p.m.	6	6.0	64	.00403	116
20 p.p.m.	5	3.7	39	.01046	299

The plants of this series showed a more definite visible injury at the three higher concentrations than did any of the preceding series, although the respiration curve is entirely similar to that of determination 3, the plants of which showed almost no injury. The data are shown in table v1 and fig. 5.

## **RESPIRATION DETERMINATION 5**

Control-Plants normal.

1 p.p.m.—Slightly less green than controls.

5 p.p.m.—Less green than the preceding; some lower leaves dropped. 10 p.p.m.—Less green than the preceding; more of the lower leaves dropped. 20 p.p.m.—Definitely chlorotic, and quite stunted; all lower leaves dropped.

The visible toxic symptoms do not correspond necessarily to the decrease in weight, as is shown by table VII and fig. 6.

### TABLE VII

RESPIRATION DETERMINATION 5, DATA TAKEN APRIL 22, 1932

Iodine concen- tration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO <sub>2</sub> output in 10 hrs. per gm. fresh wt. (gms.)	Relative CO <sub>2</sub> output per gm. fresh wt. (con- trol as 100)
Control	6	8.2	100	.00472	100
1 p.p.m.	6	6.1	74	.00450	95
5 p.p.m.	5	6.6	80	.00528	112
10 p.p.m.	5	8.7	106	.00580	123
20 p.p.m.	4	3.3	40	.01588	332

### **RESPIRATION DETERMINATION 6**

Control-Entirely normal.

1 p.p.m.—Entirely healthy and visibly larger than the controls.

5 p.p.m.-Lower leaves yellowish.

10 p.p.m.-Entire foliage yellowish; lower leaves dropped.

20 p.p.m.-More yellowish than the preceding, and more of the lower leaves gone.

Here, as in determination 3, the 1 p.p.m. plants were better than the controls, but the respiration at this point was lower as shown in table vii and fig. 7.

The average of all determinations is represented in table 1X and fig. 8. The absolute values given in the tables for the different sets of determinations are not strictly comparable. The conditions of the determinations varied as to temperature, length of the experimental period, and the relative degree of

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Fig. 7. Respiration Determination 6.

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### TABLE VIII

### RESPIRATION DETERMINATION 6, DATA TAKEN APRIL 25, 1932

Iodine concen- tration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO <sub>2</sub> output in 11 hrs. per gm. fresh wt. (gms.)	Relative CO <sub>2</sub> output per gm. fresh wt. (con- trol as 100)
Control	6	9.1	100	.00464	100
1 p.p.m.	6	11.5	126	.00246	53
5 p.p.m.	5	6.9	75	.00371	80
10 p.p.m.	6	7.0	76	.00596	128
20 p.p.m.	5	3.2	35	.01320	284
20 p.p.m.	0	0.4	00	.01020	

iodine injury. However, the conditions within any one set of determinations were identical since they were carried out simultaneously. It is for this reason that the graphs are drawn on the basis of the relative values within a given set. It is probably not justifiable to give absolute values for the averages of determinations under different conditions. The average of the relative values does give, however, a general picture of the reactions under the various sets of conditions.

## TABLE IX

AVERAGE FOR ALL RESPIRATION DETERMINATIONS

Iodine concen- tration	Number of plants averaged	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	Average CO <sub>2</sub> output in 10 hrs. per gm. fresh wt. (gms.)	Average relative CO <sub>2</sub> output per gm. fresh wt. (control as 100)
Control	36	9.2	100	.00424	100
1 p.p.m.	36	8.4	91	.00349	85
5 p.p.m.	32	6.7	73	.00463	113
10 p.p.m.	35	6.7	73	.00587	138
20 p.p.m.	31	4.9	53	.01208	282

The data obtained in this study show that at low concentrations of iodine (1 p.p.m. as potassium iodide) the respiratory activity, as represented by weight of carbon dioxide given off per gram of fresh weight over a definite time, is lowered. This lowering occurs independently of whether the plants do or do not show visible toxic effects. From this point there is a gradual recovery of normal respiratory activity which is usually reached at iodine concentrations of 5 to 10 p.p.m. The

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curve of the average in fig. 8 indicates that recovery occurs between 1 and 5 p.p.m., but this is because the average includes the erratic value represented in fig. 2 of determination 1. These results agree in general with those of Scharrer and Claus reported above.

Definitely *visible* toxic effects are evident only at the two higher concentrations, and these are the cultures showing the great increase in respiration. It should be noted, however, that



Fig. 8. Average of all respiration determinations.

the extent of the increase is not proportional to the degree of visible injury, and in some instances plants apparently healthy (although often not) show remarkable respiratory stimulation. It is also to be noted that this great increase, sometimes more than 300 per cent for the 20 p.p.m. plants, over that of the controls is a permanent respiratory level and is not the usual respiratory stimulation by toxic substances reported by many authors, which is of a temporary nature. Copeland ('03) found a close parallel between the toxicity

of various metal ions and the expulsion of carbon dioxide from the cell. He points out that this may be due to the power of metallic ions to cause the decomposition of carbonate in the

cell sap, and not therefore a stimulation of respiration. This interpretation is supported by the fact that these ions also cause an expulsion of carbon dioxide from tap water. Iodine and antipyrin did not evolve carbon dioxide from tap water, and therefore these substances may be assumed to stimulate the carbon dioxide evolution by an action on the protoplasm itself. This author mentioned that no poison has been found

that does not stimulate carbon-dioxide production.

Irving ('11) reported that the proper concentration of chloroform would stimulate respiration of barley shoots and cherry laurel. Thoday ('13) also found that chloroform stimulated respiration and moreover that the absorption of oxygen kept apace with the increased carbon-dioxide production, and therefore he concluded that the respiratory process remained properly coordinated. This was found true only at the lower concentrations. A general review of the toxic substances stimulating respiration is too extended to include here, but attention should also be called to the work of Brooks ('18), Thomas ('18), and Ray ('23 a, b, c). There is general agreement that toxic substances stimulate carbon-dioxide evolution, and consequently we may not ascribe the great increase of carbon dioxide in our plants at the higher and obviously toxic concentrations as entirely due to any specific effect of iodine. However, the fact that the increase in respiration did not parallel the extent of injury does show that the iodine has some effect aside from the general stimulation by toxic substances. Whether our results conflict with those of Stoklasa cannot be decided at this time. He reports that the sugar beets used by him were stimulated in growth as well as in the respiratory activity of the roots, and if this be true the question of toxicity would not be a factor. It is entirely possible that different species of plants react differently.

### E. OXIDIZING ENZYMES

1. Catalase.—Issajew ('05) studied the effect of hydriodic acid, potassium iodide, and of elementary iodine on preparations of yeast catalase. A concentration of N/2000 hydriodic acid completely inhibited catalase, while potassium iodide at a

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concentration of N/10 depressed the reaction very slightly, if at all. Elementary iodine was completely inhibitory in a concentration of N/1152.

Juschtschenko ('11) removed the thyroid gland of dogs, and found that the blood catalase decreased, but if thyroid preparations were fed "per os" there was a recovery. It was found even possible to increase the catalase of the blood above the

normal. The feeding of thyroid preparations to normal dogs, producing artificial hyperthyroidism, always caused an increase in blood catalase. When this feeding was discontinued, a normal condition was soon gained. Approximately the same results were obtained with rabbits. It is important to note that this author did not continue his observation of catalase activity in his test animals for a long enough time to show the effects of the accumulating amounts of iodine.

Strauss ('12) administered potassium iodide and sodium iodide to rabbits in amounts up to 4.0 grams per kilogram of body weight. The catalase activity was measured after 1, 41/2, and 6 hours. The larger doses increased the catalase activity of the blood. This increase was ascribed to the effect of the iodides on the physiology of the organism rather than to any direct effect of these compounds in the blood. Bach and Cheraskowa ('24) observed the activity of blood catalase of goats, 5 of which were normal animals and 7 had undergone thyroidectomy. Measurements were made daily for 10 days, but no difference between the two groups of animals was apparent. The most extended study of this kind is that of Timofejewa ('27). In vitro studies of rabbits' blood showed that additions of potassium iodide were without effect, while a solution of iodine as potassium iodide, even at very low concentrations, lowered the catalase activity. Artificially prepared iodized proteins also exerted a depressing effect. Experiments with commercial thyroid were also performed. Subcutaneous injections of potassium iodide alone and of iodine dissolved in potassium iodide were given daily, and the blood samples were examined every 3 or 4 days. Both types of solutions showed

a preliminary depression extending over a period of about 3 months, but this period was followed by a great increase in activity. Thyroid feeding also gave a curve of catalase activity similar to that of inorganic iodine except that the preliminary period of depression was of much shorter duration. This author concludes, from a comparison of the *in vitro* and *in vivo* experiments, that there is a relation between the thyroid

function and the activity of blood catalase.

Although the function of catalase, particularly its connection with respiratory metabolism, has not been definitely determined, its very wide distribution in plant and animal tissues postulates its great importance in living processes.

In view of the results of the animal physiologists cited above, it seemed important to carry out *in vivo* experiments in the present study. The plants used were of the same series as those used for the respiration experiments. Catalase was determined on May 1, 1932, after the plants had been growing in the respective solutions for 37 days, by the potassium permanganate method (Waksman and Davison, '26). The upper half of the plant was cut into small pieces and ground to a paste with purified quartz sand. A pinch of powdered calcium carbonate was added to neutralize the organic acids. The paste was then squeezed through two thicknesses of cheesecloth, and 1 cc. of the liquid was added to the substate and allowed to incubate at room temperature for 3 hours.

## TABLE X

CATALASE DETERMINATION 1

			$H_2O_2$	lecomposi	tion of 1 cc. of	sap
Iodine	Fresh wt.	Fresh wt.	No buf	fer	With buffer	
concent. (p.p.m.)	of tops (gms.)	(control as 100)	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control as 100	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control of "no buffer" as 100
Control	19.8	100	.005336	100	.006628	124
1	9.6	.48	.005306	99	.005952	112
5	9.4	.47	.004502	84	.005890	110
10	6.5	.33	.005888	110	.006304	118
20	6.0	.30	.007060	149	.008530	159

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### TABLE XI

### CATALASE DETERMINATION 2

			H2O2 0	lecomposi	ition of 1 cc. of	sap
Iodine	Fresh wt.	Fresh wt.	No buf	fer	With buffer	
concent. (p.p.m.)	of tops (gms.)	(control as 100)	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control as 100	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control of "no buffer" as 100
Control	10.7	100	.006424	100	.007250	113
1	6.6	62	.003700	58	.002768	43
5	9.7	91	.005090	79	.005770	89
10	7.4	69	.005638	88	.006916	108
20	10.4	101	.007444	116	.008096	126

## TABLE XII

### CATALASE DETERMINATION 3

			$H_2O_2$ d	lecomposi	tion by 1 cc. of	sap
Iodine	Fresh wt.	Fresh wt.	No buf	fer	With h	ouffer
concent. (p.p.m.)	of tops (gms.)	(control as 100)	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control as 100	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control of "no buffer" as 100
Control	10.2	100	.006420	100	.006940	108
1	5.6	55	.004880	76	.005760	89
5	6.0	59	.005658	88	.006454	101
10	7.4	73	.008778	137	.009542	149
20	9.4	92	.007700	119	.008918	139

## TABLE XIII

## CATALASE DETERMINATION 4

			H <sub>2</sub> O <sub>2</sub>	decompos	sed by 1 cc. of	sap
Iodine concent. (p.p.m.) Fresh wt. of tops (gms.)	Fresh wt.	Fresh wt.	No buf	fer	With 1	ouffer
	(control as 100)	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control as 100	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control of "no buffer" as 100	
Control	10.0	100	.007098	100	.007894	111
1	19.1	191	.005046	71	.006012	85
5	8.4	84	.005706	83	.006610	93
10	8.0	80	.006732	95	.007730	109
20	8.3	83	.008084	113	.091600	129

Parallel determinations were made on two types of substrate, one being buffered at pH 7.0, the other with no buffer. The buffered substrate was prepared by adding .0100 equivalent of hydrogen peroxide to 40 cc. of distilled water, then 5 cc. of the buffer solution. The buffer consisted of 11.6 grams of potassium-di-hydrogen-phosphate and 24.9 grams of disodium-hydrogen-phosphate per liter. Five-cc. portions added to 45 cc. of the reacting mixture maintains a pH of about 7.0. The unbuffered substrate was the same except for the absence of the buffer. The reacting substances were shaken every few



Fig. 9. Catalase Determination 1. A represents determinations on normal press juice; B, those in buffer.

### TABLE XIV

### AVERAGE OF ALL CATALASE DETERMINATIONS

			$H_2O_2$	decompos	sed by 1 cc. of	sap
Iodine concent. o (p.p.m.) (	Fresh wt.	Fresh wt.	No buffer		With buffer	
	of tops (gms.)	(control as 100)	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control as 100	Equivalents of H <sub>2</sub> O <sub>2</sub> used up	Control of "no buffer" as 100
Control	12.6	100	.006319	100	.007178	114
1	10.2	81	.004733	76	.005123	82
5	8.4	67	.005239	83	.006181	98
10	7.3	58	.006759	108	.007623	121
20	8.5	68	.007547	124	.008676	138

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minutes to insure thorough mixing. At the desired time, the reaction was stopped by adding 20 cc. of a 25 per cent sulphuric acid solution. Titration was carried out immediately with N/5 potassium permanganate.

The results (tables x-xiv and figs. 9–13 inclusive) show that the plants growing in the lower concentrations of potassium iodide exhibited a lower catalase activity of the sap than did





Fig. 10. Catalase Determination 2.

75



Fig. 11. Catalase Determination 3.

the controls, but between 5 and 10 p.p.m. of iodine, normal activity was regained. The higher concentrations always produced plants with catalase activity conspicuously greater than the controls. These results agree with those of Timofejewa cited above. It should be noted that the preliminary drop and the subsequent rise does not seem to be proportional to the degree of iodine injury as shown by the fresh weight of tops.

This is particularly noticeable in fig. 12, which shows the plant growing in 1 p.p.m. iodine as potassium iodide to be much larger than the control, yet this exceptional plant exhibited the preliminary drop just as did all the other plants growing in this type of solution.

50





Fig. 13. Average of all catalase determinations.

2. Peroxidase.—The method of Guthrie ('31) was used to determine quantitatively peroxidase and oxidase. This author points out that the mixture of alpha-naphthol and paraphenylenediamine is oxidized far too rapidly for quantitative determination of these enzymes if the mixture is near neutrality, but if the reacting solution be buffered at pH 4.5, the rate of oxidation is sufficiently slow to allow accurate comparisons to be made. This acidity also inhibits catalase and prevents the interference of this enzyme. The citrate buffer was made by dissolving 21 grams of

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crystalline citric acid in 170 cc. of normal sodium hydroxide, and diluting to 1 liter. The substrate was prepared as follows: to 200 cc. of the citrate buffer, 200 cc. of water was



added, and then 1 gram of para-phenylenediamine hydrochloride and 20 cc. of a 4 per cent solution of alpha-naphthol in 50 per cent alcohol. This was filtered and used immediately.

The upper portions of the plants were ground with purified quartz sand, and the mass squeezed through cheese-



Fig. 18. Average of all peroxidase determinations.

cloth. Portions of 1 cc. were added to 25 cc. of the freshly prepared substrate. This was followed by the addition of 5 cc.

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of N/20 hydrogen peroxide. After 15 minutes the reaction was stopped by the addition of 5 cc. of a .1 per cent aqueous solution of potassium cyanide. Twenty-five cc. of toluene were then added and shaken vigorously until the water layer appeared colorless. The toluene, containing the indophenol,

### TABLE XV

### PEROXIDASE DETERMINATION 1

Tadina anneat			Relative peroxidase activity		
(p.p.m.)	tops (gms.)	trol as 100)	Determin. A (control as 100)	Determin. B (control as 100)	
Control	27.7	100	100	100	
1	18.2	69	109	114	
5	13.5	49	128	119	
10	12.7	46	194	217	
20	9.1	33	167	149	

## TABLE XVI

#### PEROXIDASE DETERMINATION 2

Tadina agent			Relative peroxidase activity		
(p.p.m.)	tops (gms.)	rresh wt. (con- trol as 100)	Determin. A (control as 100)	Determin. B (control as 100)	
Control	18.3	100	100	100	
1	17.5	95	83	92	
5	12.6	69	118	127	
10	10.7	53	200	189	
20	8.1	44	244	279	

### TABLE XVII

### PEROXIDASE DETERMINATION 3

Taling			Relative peroxidase activity		
(p.p.m.)	tops (gms.)	trol as 100)	Determin. A (control as 100)	Determin. B (control as 100)	
Control	17.9	100	100	100	
1	12.5	69	89	89	
5	14.7	82	96	98	
10	12.6	71	139	143	
20	6.1	48	167	156	

### TABLE XVIII

#### PEROXIDASE DETERMINATION 4

			Relative peroxidase activity		
Iodine Concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (con- trol as 100)	Determin. A (control as 100)	Determin. B (control as 100)	
Control	17.6	100	100	100	
1	11.3	64	94	87	
5	7.7	44	74	72	
10	11.2	64	127	135	
20	7.2	41	149	147	

### TABLE XIX

### AVERAGE OF ALL PEROXIDASE DETERMINATIONS

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	Relative peroxidase activity (control as 100)
Control	20.4	100	100
1	14.9	74	95
5	12.1	61	105
10	11.8	59	168
20	7.6	42	182

was then poured off, centrifuged, and examined in a colorimeter. Samples from the entire series of plants were run simultaneously and in duplicate.

The oxidase determinations were carried out identically and simultaneously on the same samples of press juice, but with the omission of the hydrogen peroxide.

Since comparative results, rather than absolute amounts, were the object of the study, the intensity of color of the centrifuged toluene-indophenol solution obtained from the action of the sap of the control plants was taken as 100, and the other values calculated comparatively. The determinations were made on May 8, 1932, after the plants had grown in the respective solutions for 44 days.

The results are set forth in tables xv to xix, and figs. 14 to 18 inclusive. Here again there is a preliminary period of depression, followed by a significant increase. A comparison of the growth curves with the enzyme curves shows that the

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increase of peroxidase activity is not proportional to the degree of injury by the toxic concentrations of potassium iodide. The curves do, however, closely parallel those of respiration.

3. Oxygenase.—The terms oxidase and oxygenase have been variously used in the literature. Bach and Chodat defined oxygenase as an organic peroxide. Kostytschew ('26) states that, "Die unbeständigen Substanzen vom Peroxydtypus führen den Namen Oxygenasen. Ihre chemische Natur ist bis jetzt unbekannt, doch sind sie allem Anschein nach organische Substanzen. Die reduzierten Oxygenasen, die den labilen Saurerstoff abgespalten haben, nennt man Oxydasen. Die Oxydasen gehen unter Sauerstoffaufnahme wieder in Oxygenasen über." Other authors have used oxidase to denote the oxygen-activating enzyme and oxygenase as the complex of oxidase plus the quinone-like substances produced by it. This complex has the ability to oxidize many substances and has therefore been loosely referred to as oxygenase. In the present work, the term oxygenase is defined as the enzymatic catalyst by which molecular oxygen is activated toward substances of the general catechol type. As indicated above, the determinations of oxygenase activity were carried out like those of peroxidase except that hydrogen peroxide was omitted from the reaction mixture. Theoretically, this should be a satisfactory means of determining oxidase activity, but as has been pointed out by Onslow ('31), the problem is complicated as shown in the following paragraphs. Szent-Györgyi ('25), in his study of oxidation mechanisms of the potato, determined that compounds of the ortho-quinone type were formed by the action of oxygenase on compounds having the ortho-dihydroxy-benzene grouping. These orthoquinones are to be considered as the final product of the action of oxygenase itself. During this process, peroxide is formed: (1) either by the preliminary formation of organic (catechol) peroxides by a union with free oxygen of the air, and the subsequent breaking up to give hydrogen peroxide and some oxidation product of catechol; or (2) more directly by the

transference of hydrogen from catechol compounds to molecular oxygen producing hydrogen peroxide and orthoquinone.

In the present oxygenase studies 1-cc. aliquots of the same expressed plant juice as used for the peroxidase determination were taken, and both enzymes determined simultaneously. Since peroxidase was present in large amounts, particularly in the plants grown in the higher potassium-iodide concentrations, there is no reason why the peroxide formed by oxygenase could not have initiated reactions giving the same oxidation products as the oxygenase. This is undoubtedly a source of error in the present work, since a part of the color developed (indo-phenol) came from this side reaction between peroxidase and the developing peroxide. If, however, these reactions seriously affected the results, we could expect the plant juice having the greatest peroxidase to give also the greatest apparent oxygenase reaction. From a comparison of the curves for these two enzymes it may be seen that this is not the case. The significance of this source of error is further lessened by the fact that some of the peroxide acted upon by peroxidase originated from the oxygenase reactions. For this reason it is seen that while more indo-phenol may have been formed, the increase was proportional to that of oxygenase activity, and the general shape of the curve would be the same. The results of the present study are set forth in tables xxxxiv, and figs. 19-23. While the individual curves vary considerably the average (fig. 23) shows a small but progressive decrease of oxygenase activity in the plants grown in the higher potassium iodide solutions. This is particularly significant since the source of error discussed above tends to push the curve up, rather than down. It should be emphasized again that aliquots of these same samples of press juice show simultaneously a great peroxidase increase.

Owing to the comparatively slight variation of the amount

of oxygenase activity the question might be raised as to whether this enzyme was present at all, especially since no absolute values are given. Onslow ('21) has determined that

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only about 60 per cent of the higher green plants contain the catechol-oxygenase system. Tomato fruit is known, moreover, to contain no oxygenase. In the controls of the reagents alone, carried out simultaneously with the oxygenase determination, a little indo-phenol was developed through atmospheric oxidation, but in no case did this amount ever exceed 16 per cent of that found in the experimental solutions. Hence

the presence of oxygenase in the vegetative portion is clearly



Fig. 20. Oxygenase Determination 2.

### TABLE XX

OXYGENASE DETERMINATION 1, OF THE SAME PLANT JUICE AS PEROXI-DASE DETERMINATION 1

<b>T D</b>			Relative oxygenase activity		
(p.p.m.)	tops (gms.)	(control as 100)	Determin. A (control as 100)	Determin. B (control as 100)	
Control	27.7	100	100	100	
1	18.2	69	118	115	
5	13.5	49	121	115	
10	12.7	46	108	102	
20	9.1	33	107	91	

### TABLE XXI

OXYGENASE DETERMINATION 2, OF THE SAME PLANT JUICE AS PEROXI-DASE DETERMINATION 2

			Relative oxygenase activity		
Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Determin. A (control as 100)	Determin. B (control as 100)	
Control	18.3	100	100	Determin. lost	
1	17.5	95	104	Determin. lost	
5	12.6	69	92	Determin. lost	
10	10.7	53	97	Determin. lost	
20	8.1	44	94	Determin. lost	

### TABLE XXII

OXYGENASE DETERMINATION 3, OF THE SAME PLANT JUICE AS PEROXI-DASE DETERMINATION 3

			Relative oxygenase activity		
Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Determin. A (control as 100)	Determin. B (control as 100)	
Control	17.9	100	100	100	
1	12.5	69	80	83	
5	14.7	82	80	81	
10	12.6	71	77	82	

20	6.1	48	68	79

### TABLE XXIII

#### OXYGENASE DETERMINATION 4, OF THE SAME PLANT JUICE AS PEROXI-DASE DETERMINATION 4

			Relative oxygenase activity			
lodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Determin. A (control as 100)	Determin. B (control as 100)		
Control	17.6	100	100	100		
1	11.3	64	63	91		
5	7.7	44	56	71		
10	11.2	64	68	92		
20	7.2	41	57	73		

demonstrated. Bunzell ('16) also found oxygenase activity in the tomato plant. Two cc. of juice expressed from the leaves absorbed sufficient atmospheric oxygen to reduce the

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### TABLE XXIV

### AVERAGE OF ALL OXYGENASE DETERMINATIONS

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Relative oxygenase activity (control as 100)
Control	20.4	100	100
1	14.9	74	93
5	12.1	61	88



25 1



Fig. 22. Oxygenase Determination 4.



Fig. 23. Average of all oxygenase determinations.

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pressure in his apparatus 1.10–1.60 mm. of mercury. The substrate used was of the catechol type, and could therefore give the indo-phenol reaction by the intermediate formation of quinones.

The significance of the oxygenase-catechol system in plants has been much discussed. Palladin's theory, that respiratory chromogens, e.g., catechol-oxygenase or similar systems, furnish a mechanism for respiratory oxidation, depends upon this enzyme. This position is materially weakened by the fact that in the tissues of only about one-half of the higher plant orders may such a system be recognized. However, Keilin ('29) held the respiratory function of such an oxidation mechanism to be conclusively proved for yeast and animal tissues. This author used the same reagents and approximately the same procedure that was used in the present work. Onslow ('31) believes that an effective mechanism would be more universally distributed in tissues, and therefore the existence of the catechol-oxygenase system should be regarded as aside from the fundamental respiratory mechanism. This author also emphasized experimental results, which show that only plants containing the substrate, e.g., catechol or similar compounds, contain oxygenase; hence the real problem is that of the physiology of the formation of these substances rather than their connection with oxygenase. A comparison of the respiratory with the oxygenase curves would indicate that there is no connection in the tomato plant between respiration activity as shown by carbon-dioxide output and this enzyme. On the other hand, the peroxidase curves do show such a relationship. The author therefore believes it logical to assume that respiration is being carried on in some manner entirely separate from the catecholoxygenase system. It then follows that if the comparatively small decrease of oxygenase activity in the plants grown in solutions of higher concentrations of potassium iodide be considered significant, it must be assumed that the increasing iodine content of the tissue has interfered with the normal physiology of the production of the ortho-dihydroxy-benzene

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type of compounds. It is greatly regretted that the lack of sufficient tissue prevented an actual quantitative study of these substances.

## F. NON-OXIDIZING ENZYMES

1. Invertase.—The effect of iodine and potassium iodide on the activity of invertase has been carefully studied in vitro. Euler and Landergren ('22) were the first to attack this phase of iodine physiology. They added a solution containing .74 mgm. of elemental iodine and 1.50 mgms. of potassium iodide per cc. to their invertase preparation and held it at 17° C. for one hour. The preparation was then added to the sugar solution containing 4.8 gms. of sucrose and 2 per cent phosphate, giving a total volume of 60 cc. These authors found that the concentration of potassium iodide used (.01 M) did not materially inactivate the invertase. The relative inversion velocity was 58.7 per cent of the control when .07 mgm. of elemental iodine was present. The relative inversion velocity was 45.9 per cent of the control in the presence of 10 times (.74 mgm.) this amount of iodine, thus the decrease of invertase activity was small compared to the wide range of iodine concentrations. It was concluded that while invertase was sensitive to iodine, most of its activity remained stable, even with large amounts of this substance. Treatment with sodiumthio-sulphate did not reactivate the enzyme. The work was continued by Euler and Josephson ('22, '23). They considered that the invertase formed an iodine-invertase compound and they attempted its isolation and also to complete its inactivation by a silver salt. They found that the degree of inactivation by iodine and potassium iodide mixture was dependent on the time during which the iodine acts on the enzyme, and called this the "incubation" time. Complete inactivation by iodine was accomplished by lengthening the period of incubation. It is not yet certain that the inactivation depends upon any stoichiometric relation. There is also the possibility that iodine catalyzes the inactivation and therefore does not appear as an end product. Hence it might in-

activate a larger number of invertase molecules than would correspond to its own number of molecules. Their study of the reaction constant of the inactivation process tends to support this assumption. These workers also found that while increasing amounts of iodine did not inactivate the invertase beyond a certain point, if the incubation time were short, bromine water could obviously call forth any degree of inactivation more or less independent of the incubation time, the degree of inactivation depending upon the bromine content. In summing up their work, Euler and Josephson made the following statements concerning the relation of invertase to iodine:

1. Iodine poisoning is dependent upon the time of incubation.

2. Iodine appears to exert a stronger effect on invertase of high purity than does bromine.

3. One gram of iodine suffices, under the conditions used, to depress the activity of 20,000 grams of invertase, of an initial inversion power of If = 230, to about one-half.

4. The 50 per cent inhibited enzymes show the same optimal pH as the control.
5. The 50 per cent inhibited enzyme is held to be an iodine-invertase compound which is also an enzyme but of lower inversion power.

6. Bromine and silver agree very closely in their ability to inactivate invertase, but do not agree with the iodine inactivation equivalent. This indicates a similarity of the reaction of invertase to silver and bromine, but a specific and different reaction to iodine.

The present investigation of the effect of potassium iodide upon the invertase activity was carried out *in vivo*. Tomato plants grown in nutrient solutions containing increasing amounts of potassium iodide were harvested after a growth period of 45 days. The tops were cut in small pieces and dried in absolute alcohol and washed in ether and then spread out to dry. The dried material was then ground to a fine powder. One gram of the dry powder was mixed with 100 cc. of distilled water and soaked 12 hours in the Kelvinator. Then 15 grams of commercial sucrose were added and the mixture incubated at  $37^{\circ}$  C. for 24–48 hours. After incubation, the mixture was filtered and 10-cc. aliquots analyzed for reducing sugars. To the 10-cc. aliquots 15 cc. distilled water and 30 cc. of Fehling's solution were added. The 5 solutions to be com-

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pared were simultaneously boiled 3 minutes in large test-tubes in the same beaker of water. This insured comparable conditions of reduction in the different types of plants, and since

125 100.



Fig. 25. Invertase Determination 2.

the final values are reported as per cent of the control, the absolute amount of reduction in any one series becomes unimportant. The copper oxide was immediately filtered off and dissolved in Bertrand's solution (20 per cent sulphuric acid which had been saturated with ferric-sulphate) as recom-

mended by Morris and Wesp ('32). The amount of reduced iron in the acid solution was titrated with N/20 potassium permanganate.

150 125



æ

Invertase Determination 4. Fig. 27.

The number of cubic centimeters of permanganate used was taken as the basis for calculating the results comparatively. The results are shown in tables xxv-xxix and in figs. 24-28. As is the case with the previous enzymes studied, with the ex-

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ception of oxygenase, the preliminary period of depression is followed by a great increase. The effect of iodine in the previous *in vitro* studies is seen to be very different from the present *in vivo* experiments. This further supports the findings of Fuller ('32), who stated that enzymes behaved very differently toward ultra-violet radiation in his *in vivo* and *in vitro* studies.

![](_page_41_Figure_3.jpeg)

Onslow ('31) reviews the literature dealing with the substrate for respiration in the higher green plants. In studies made on the change of carbohydrate fractions of respiring organs she noted that a hexose sugar is the substrate for respiration, and further, that fructose from sucrose hydrolysis

### TABLE XXV

Iodine		cc. KMn	O4 used	Relative amt. KMnO, used (control as 100)				
in p.p.m.	Set 1	Set 2	Set 3	Ave.	Set 1	Set 2	Set 3	Ave
Control	7.61	9.17	8.58	8.45	100	100	100	100
1	6.98	8.56	6.73	7.42	92	93	78	88
5	7.67	9.22	7.70	8.20	101	101	89	97
10	8.22	10.82	9.15	9.40	108	118	107	111
20	17.20	19.16	17.02	17.79	226	209	198	210

INVERTASE DETERMINATION 1, INCUBATION PERIOD 24 HOURS

### TABLE XXVI

### INVERTASE DETERMINATION 2, INCUBATION PERIOD 24 HOURS

Iodine		cc. KM1	10, used	Relative amt. KMnO, us (control as 100)				
in p.p.m.	Set 1	Set 2	Set 3	Ave.	Set 1	Set 2	Set 3	Ave.
Control	10.92	10.45	9.22	10.20	100	100	100	100
1	8.30	8.54	8.25	8.36	76	82	89	82
5	11.66	11.21	9.92	10.93	107	107	107	107
10	12.07	13.16	12.05	12.43	111	125	129	122
20	25.57	26.72	21.61	24.63	234	255	234	241

### TABLE XXVII

#### INVERTASE DETERMINATION 3, INCUBATION PERIOD 40 HOURS

Iodine		cc. KMr	O <sub>4</sub> used	Relative amt. KMnO, used (control as 100)				
in p.p.m.	Set 1	Set 2	Set 3	Ave.	Set 1	Set 2	Set 3	Ave.
Control	12.28	17.27	14.46	14.67	100	100	100	100
1	9.46	12.59	9.05	10.37	77	73	62	71
5	11.71	15.04	12.54	13.10	95	87	86	89
10	15.08	16.67	16.72	16.16	123	97	115	112
20	30.89	35.12	31.68	32.56	251	203	219	224

### TABLE XXVIII

#### INVERTASE DETERMINATION 4, INCUBATION PERIOD 48 HOURS

Iodine		cc. KMI	nO₄ used	Relative amt. KMnO, used (control as 100)				
in p.p.m.	Set 1	Set 2	Set 3	Ave.	Set 1	Set 2	Set 3	Ave.
Control	15.14	17.05	14.80	15.69	100	100	100	100
1	8.78	12.85	9.69	10.44	58	75	65	66
5	10.56	15.30	12.51	12.79	69	89	84	81
10	11.24	16.16	13.36	13.56	81	95	89	88
20	30.38	34.70	30.89	31.79	201	203	208	204

is preferentially used. Should this fructose be exhausted, the more stable glucose may be utilized. To quote only one of many available studies concerning this problem, Evans ('28) found 80 per cent of the total hexose in stored apples to be glucose, the fructose from disaccharide hydrolysis having been depleted through respiration.

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### TABLE XXIX

AVERAGE OF ALL INVERTASE DETERMINATIONS, THE CONTROL TAKEN AS 100

Iodine concent. in p.p.m.	Relative invertase activity
Control	100
1	77
5	94

10 20 20

Since it is well authenticated that a hexose sugar serves as the respiratory substrate, it is to be expected that a conspicuous increase in invertase activity would accompany accelerated respiration. This is based on the concept discussed later that sucrose normally predominates over hexose in the tomato plant. Wehmer ('31) summarizes the analytical results of many publications concerning the sugar content of the fruit. The total sugar has been variously reported as being 3–5 per cent of the fresh weight. Sucrose has been reported as being about 1.7 per cent, levulose 1.12 per cent and glucose 1.12 per cent. Unfortunately, no published analyses have come to our notice concerning the carbohydrate fractions of the vegetative tissue.

2. Peptase.—Peptase, being a plant enzyme not directly concerned with the respiratory process, was used in order to determine whether iodine would affect it differently from those involving respiratory metabolism. The method of procedure was that of Fisher ('19) The number of free carboxyl groups was estimated by the method of Sörensen ('08).

The plants were grown in the culture solutions for 43 days. The tops were then shredded and dried by alcohol and washed with acetone as described for the material used for the invertase determinations. One gm. of dry plant powder was added to 200 cc. of a 2 per cent Witte peptone solution, and the mixture plus a little toluol was incubated for 4 days at 37° C. After that time, the material was filtered and the residue washed until the filtrate came to 400 cc. The filtrate was de-

colorized by shaking with alumina cream. Aliquots of 40 cc. of the prepared solution were titrated in the following manner. First, 15 cc. of a formaldehyde mixture were added. This solution was made by mixing 50 cc. of commercial formalin (40 per cent) with 25 cc. of absolute alcohol, and then adding 10 cc. of indicator. The indicator was prepared by dissolving .50 gram of Grubler's thymol-phthalein in 1000 cc. of 93 per cent alcohol. The free amino groups of the liberated amino acids react as a base, thereby masking the carboxyl groups. Hence formaldehyde is used to neutralize those groups by the formation of methylene compounds. The free carboxyl groups were then titrated by N/5 barium hydroxide.

![](_page_44_Figure_2.jpeg)

The number of cubic centimeters of hydroxide used is taken as the basis for comparison. The results are shown in tables xxx and xxx1 and in fig. 29. The figure shows a very small preliminary drop, followed by a very small rise in peptoclastic activity. It is probable that these changes are not significant in view of possible inaccuracies of the method.

### TABLE XXX

#### PEPTASE DETERMINATION 1

Iodine		cc. Ba(OH	(), used	Comparat	ive amoun (control a	nt Ba(OH), used as 100)		
p.p.m.	Titrat. 1	Titrat. 2	Titrat. 3	Ave.	Titrat. 1	Titrat. 2	Titrat. 3	Ave.
Control	10.5	10.7	10.7	10.6	100	100	100	100
1	10.0	9.9	10.1	10.0	95	92	94	94
5	10.8	11.0	10.9	10.9	103	104	102	103
10	10.3	10.7	10.7	10.6	98	100	100	100
20	11.5	11.7	11.7	11.6	109	109	109	109

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### TABLE XXXI

#### PEPTASE DETERMINATION 2

Iodine conc. in		cc. Ba(OH	() <sub>2</sub> used	Comparat	t Ba(OH) is 100)	2 used		
p.p.m.	Titrat. 1	Titrat. 2	Titrat. 3	Ave.	Titrat. 1	Titrat. 2	Titrat. 3	Ave.
Control	11.4	10.4	10.2	10.7	100	100	100	100
1	10.7	10.0	9.9	10.3	94	96	97	96
5	10.9	11.2	11.3	11.1	96	108	111	104
10	11.0	10.6	10.5	10.7	97	102	103	100
20	11.2	11.0	11.5	11.2	98	106	112	105

IV. THE RELATIONSHIPS BETWEEN OXYGENASE, PEROXIDASE, INVERTASE, AND CATALASE

A. RESPIRATORY PIGMENTS

The theory of the function of respiratory pigments has been elaborated at some length by Palladin ('08-'12), Palladin and Lwow ('13), and by Palladin and Tolstoi ('13). These workers considered that respiratory pigments accept hydrogen from biologically oxidizable material and transfer it to the oxygen of the air, the pigments thereby becoming reduced to chromogens. This theory would regard biological oxidation as essentially a dehydrogenation process. Other students of respiratory mechanism have placed a varying degree of importance on this interpretation. Bach, Thunberg, and Wieland each regard the transfer of hydrogen of great significance. Several specific carriers of hydrogen have been identified from time to time. The glutathione of Hopkins ('21 a, b), the cytochrome of Keilin ('29), the hexuronic acid of Szent-Györgyi ('31), and the blue pigment found in Bacillus pyocyaneus by Friedheim ('31) are examples.

The fact that methylene blue has been found by many workers to stimulate metabolism is evidence of the importance of a dehydrogenating system for respiration. Warburg, Kubowitz and Christian ('30 a, b) have determined the mechanism by which methylene blue accelerates respiration. They interpret the reaction by arranging the following three equations:

Methylene blue + n-hemoglobin + nH<sub>2</sub>O → leuco methylene blue + n-methemoglobin.
n-methemoglobin + glucose → n-hemoglobin + glucose oxidation products + nH<sub>2</sub>O.
Leuco methylene blue + H<sub>2</sub>O<sub>2</sub> → methylene blue + H<sub>2</sub>O.

These authors did not determine whether the sugar entered the reaction as glucose, phosphoric esters, or as some hexose cleavage product. The final reaction by which the leuco compound is oxidized to the colored state is defined as a "balancing" reaction, running parallel to the cellular catalytic processes, but the actual oxidation of the carbohydrate is accomplished by hemin iron.

Eddy ('31) found that intravenous injections of methylene blue increased the oxygen uptake of the organism.

B. OBJECTIONS TO THE DEHYDROGENATION THEORY OF

## BIOLOGICAL OXIDATION

The chemical reactions by which hydrogen may be removed from oxidizable materials appear to depend upon the action of enzymes, at least in most instances. The relation between respirational reaction and enzymes has been seriously questioned by some workers. Engler and Herzog ('09) maintain that the importance of enzymes in biological respiration is greatly overrated. The fact that oxygenase and peroxidase catalyze only the reactions which account for the formation of water by the union of atmospheric oxygen and derived hydrogen and do not break carbon chains, is a serious objection to a close association of these enzymes with respiration. At least this would tend to place the oxidizing enzymes in some subsidiary relation to a breaking of the carbon chain. Bertrand ('96), Portier ('97), and Porodko ('04) placed so much importance on the fact that oxidizing enzymes could not unite oxygen to carbon to form carbon dioxide that they denied that these enzymes could attack any important respirational substrate to be found in tissue.

However, the case for oxidizing enzymes has been materially

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strengthened by the work of Lyon ('23, '27). This worker found that an aqueous extract of oxidase from potato tubers in the presence of sodium or potassium phosphate would slowly oxidize glucose or fructose with the liberation of carbon dioxide. The phosphate ion apparently acted as an intensifier of the oxidase. Wurmser ('32) aptly points out the probability of a dehydrogenating system coexisting with some sys-

tem capable of breaking carbon chains.

## C. CHEMICAL REACTIONS OF THE OXIDATION MECHANISM STUDIED IN THE PRESENT WORK

As pointed out above, the experimental substrate for the determinations of peroxidase and oxygenase consisted of a mixture of para-dimethyl-phenylene-diamine-hydrochloride and alpha-naphthol in an alcoholic solution. This differs from the original "Nadi" reagent in that it was buffered at a higher acidity to suppress the action of catalase and to slow down the rate of oxidation.

The steps of the reaction may be pictured as follows. The resulting quinone is to be considered as the final oxidation product of oxygenase itself. The reaction mixture may be assumed to contain compounds of the orthodihydroxy type since Onslow ('31) found these substances always present in plants exhibiting oxygenase activity.

1. a 
$$O_{-OH} + O_2 + \text{oxygenase} \rightarrow \text{organic peroxide} = OH$$

b Organic peroxide + 
$$H_2O \rightarrow \bigcirc = 0 + H_2O_2 = 0 = 0$$

Or the initial step may form H<sub>2</sub>O<sub>2</sub> directly:

2. 
$$\bigcirc -\text{OH} + \text{O}_2 + \text{oxygenase} \rightarrow \text{H}_2\text{O}_2 + \bigcirc = 0$$

![](_page_47_Picture_11.jpeg)

![](_page_47_Picture_12.jpeg)

The quinone reacts upon the artificial substrate, forming the colored product.

The indo-phenol may be considered as an artificial hydrogen acceptor. Such a compound in living tissue accepts hydrogen from the respirational substrate by the action of the dyhydrases.

Equations 3a, b, are those postulated by Rohmann and Spitzer ('95).

![](_page_48_Figure_3.jpeg)

If quinone be considered as the final product of oxygenase, equation 3a would be modified as follows:

![](_page_48_Figure_5.jpeg)

Since equation 3b is not dependent upon enzymatic catalysis, the leuco compound formed in 3c would autoxidize in the presence of atmospheric oxygen independently of quinone. It is not

impossible that the para-phenylene-diamine might become oxidized to the nitroso stage, in which case it would condense with the  $\alpha$ -naphthol to give the colored indo-phenol as follows:

![](_page_49_Figure_0.jpeg)

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Keilin ('29) distinguishes between his indo-phenol oxidase and the catechol oxidase of other workers. He describes indophenol oxidase as being insoluble, while catechol oxidase is soluble in water. They are both inhibited by potassium cyanide, hydrogen sulphide, and by carbon monoxide. Catechol oxidase is more sensitive to the inhibitory effect of carbon monoxide than indo-phenol oxidase. The catechol oxidase was described by him as having "all the essential properties of a typical oxidase."

It does not seem possible or necessary to separate these two oxidases in a discussion of the function of oxygen-activating enzymes in their relation to respiration. Any essential difference between these substances depends upon the assumption that indo-phenol oxidase oxidizes directly the aromatic amino groups of para-phenylene diamine or the leuco form of indophenol by the activation of atmospheric oxygen, while catechol oxidase acts only upon poly-hydroxy-benzene compounds, producing quinones, which then oxidize the leuco-indo-phenol or some naturally occurring chromogen. Keilin states that catechol oxidase will give the indo-phenol test in the presence of catechol compounds. According to Onslow ('31), this oxidase occurs only with the simultaneous occurrence of this substrate. Hence it is evident that the catechol oxidase in tissue preparations will always give the indo-phenol reaction unless great care has been used to remove all traces of polyphenolic compounds. On the other hand, in order to maintain the separate identity of these enzymes, it would be necessary to

establish the fact that indo-phenol oxidase acts upon leuco indophenol or similar naturally occurring chromogens without the intermediate cooperation of such substances as the quinones.

This would be difficult to accomplish, since leuco indo-phenol is easily autoxidizable. It does not appear from an examination of the literature that such a demonstration has been made. For demonstrating the oxidase of yeast cells, Keilin recommends a neutral 1 per cent solution of para-phenylenediamine-hydrochloride, or the familiar "Nadi" reagent consisting of equal parts of M/100 solutions of di-methyl-paraphenylene-diamine-hydrochloride and alpha-naphthol in 50 per cent alcohol and 125 per cent aqueous sodium carbonate. These reagents by definition would demonstrate the presence of the indo-phenol oxidase. It is very important to note that Tolomei ('96), Grüss ('01), and Issajew ('04) also found an oxidizing enzyme in yeast by the use of various polyphenols. This oxidase by definition would be a catechol oxidase. Happold ('30) has studied the ability of bacteria to oxidize polyphenols and dimethyl-para-phenylene-diamine. Positive tests with these two reagents would correspond to Keilin's catechol oxidase and indo-phenol oxidase respectively. Positive oxidase reactions were found for eleven different bacteria. Of these, only Staphylococcus albus failed to give the oxidase reaction

with catechol and the para-phenylene-diamine.

The work of Cook, Haldane and Mapson ('31) shows that it is entirely probable that different oxidases may exist in the cell. These workers studied the effect of carbon monoxide on the oxidation of various substances by *Bacillus Coli*, and concluded that the cell contains a number of oxidases differing from one another as do the different hemoglobins.

As pointed out above, Bunzell ('16) found an oxidase in tomato juice which acted upon pyrogallol and was therefore a catechol oxidase. In the present work the presence of an indo-phenol oxidase was determined by the use of the modified "Nadi" reagent. It is possible and probable that the catechol enzyme was the active catalyst in both cases.

The essential consideration in evaluating the work of Keilin

and of other workers who postulate a great importance for the oxidases in respiration is that the hydrogen acceptor—the chromogens of Palladin, the respiration ferment of Warburg,

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the cytochromes of Keilin, etc-is oxidized by their activity, and may then function again as a hydrogen acceptor. With this view, it is not necessary to separate the indo-phenol oxidase of Keilin and the catechol oxidase of other workers.

D. POSSIBLE ENZYMATIC SYSTEMS

Oxygenase-catechol-catalase.—Considering the indo-phenol

as an artificial respiratory pigment we may arrange the following oxygenase-catechol-catalase system. In all reactions in which indo-phenol occurs, it is intended to figuratively represent all hydrogen acceptors which have been postulated by other workers and which are reversibly oxidized and reduced. Oxygenase produces quinone and peroxide by equations 1 and 2.

The quinone restores the chromogen to the pigment. The power of quinones to accomplish this has been verified in the present work by in vitro experiments:

![](_page_51_Figure_7.jpeg)

The catechol compound is restored to quinone by equations 1 and 2.

The pigment accepts hydrogen from the respirational substrate (RH<sub>2</sub>) by the action of dehydrases:

![](_page_51_Figure_10.jpeg)

![](_page_51_Picture_11.jpeg)

The above system would be theoretically self-perpetuating. The toxic effect of the accumulating hydrogen peroxide could

be prevented by catalase. In such a case catalase would be intimately associated with the respirational process and might be expected to fluctuate more or less proportionally with it. Zaleski and Rosenberg ('11), Appleman ('18), Crocker and Harrington ('18), Burge ('20), and many others have sought to establish a relationship between this enzyme and respiration. Since it is assumed that catalase releases only molecular oxygen from hydrogen peroxide it would be difficult to account for the direct value of this additional oxygen for respirational processes in the presence of an abundance of atmospheric oxygen. If, however, the oxygenase-catechol system is accepted as an effective oxidation mechanism, then catalase would bear a necessary but indirect relation to the respiratory processes.

Oxygenase-catechol-peroxidase.—It is also possible to set up a series of equations showing an oxygenase-catechol-peroxidase system. The chromogen formed by reaction 6 might be restored by the action of peroxidase and the peroxide formed by reactions 1 or 2.

![](_page_52_Figure_3.jpeg)

This would not, of course, prevent the chromogen being oxidized also by equation 5. By this system it is possible to account not only for the lack of proportionality between catalase and respirational intensity as has been reported by Ranjan

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and Mallik ('31), Rhine ('24), Harvey ('24), and McLeod and Gordon ('23), but also to explain the coordinate rise of both oxygenase and peroxidase in the heightened respirational rate of plants suffering from disease. A correlation between the oxygenase and peroxidase under pathological condition has been often reported. Suzuki ('00, '02 a) observed this condition in the mulberry dwarf disease in Japan; Woods ('02) reported it for the mosaic disease of tobacco; and Reed ('12) found that the extract from apples infected with bitter-rot (*Glomerella rufomaculans*) showed an increased activity of these enzymes. Doby ('11, '12) observed the same condition in potato tubers infected by the Rosette disease.

Invertase-peroxidase.—A third relation may be also postulated which involves a relationship between invertase and peroxidase. Since this theory places great importance on the recent work of Ranjan and Mallik ('31), their work will be reported in some detail. They found that the edible pea during germination exhibits a gradual rise of respiration, while the catalase falls slightly at first, then rises to a maximum, and again falls. The leaves of Magnifera indica showed a parallel decrease in respiration and catalase until yellowing set in. At this time catalase increased greatly, showing that the correlation existed only in the younger stages. The same results were found with the leaves of Eugenia jambolana. An analysis of the sugar content of the leaves of both these species showed that the curves of monosaccharide content almost exactly paralleled those of catalase activity. In order to ascertain whether the sugar controlled the catalase or the catalase controlled the sugar, the stems of Allium tuberosum were injected with sugar, and some of the plants put in the light and others in the dark. The following important relation was discovered. It was not the hexose presence but the formation of hexose, either by photosynthesis or by the hydrolysis of disaccharides, which correlated with a high catalase activity. The injection of cane sugar accompanied a greater catalase activity than did glucose, although the amount of glucose present in the former case was related to the amount of catalase.

The apparent correlation between catalase and respiration as reported by other authors is explained by the fact that normally the formation of monosaccharides parallels respirational activity. Hexoses are being formed in old leaves since the complex carbohydrates are being broken down for translocation, and this accounts for the increased catalase at this stage. It is further pointed out that high catalase may be associated with low respiration unless high respiration is associated with the formation of hexose. The present author proposes to relate the above phenomenon to account for the existence of an invertase-sugar-peroxidase respirational mechanism in the tomato plant under the conditions of the experiment. Waldschmidt-Leitz ('29) points out that the only known substrate for catalase is hydrogen peroxide. Since this substance is not demonstrated in tissues, it follows that the action of catalase or peroxidase uses it as rapidly as formed. The work of Ranjan and Mallik may therefore be interpreted as indicating that hydrogen peroxide, or a peroxide group resembling it but not yet recognized, develops during the formation of hexose. This is particularly evident, since it has been widely observed that an enzyme develops more or less proportionally to the concentration of its substrate. Rhine ('24) believed that catalase developed in plant tissue proportionally to the need of the plant to overcome the toxic effects of hydrogen peroxide. Without entering the discussion concerning the importance of the ratio of sucrose to hexose in plant tissue in its relation to photosynthesis, we may note that sucrose often accumulates in a great excess over hexose. Davis and Sawyer ('16) studied the potato, a plant closely related to the tomato, and observed that sucrose was greatly in excess of hexose, and concluded that it was therefore the first sugar of photosynthesis. The same relationship has been found in many plants. Regardless of whether sucrose is or is not the first sugar of photosynthesis, it is important for our hypothesis to note its predominance over hexose in plants closely related to the tomato. Onslow ('31) reviewed the literature concerning the substrate for respiration, and shows that most of the data

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support the belief that a hexose, probably fructose, is this substrate. An increased rate of respiration would therefore call forth an increased invertase action to produce the hexose from sucrose. Since catalase has been shown to parallel the formation of hexose, it may be assumed that a transitory peroxide also develops simultaneously. Peroxidase might well use the peroxide then formed in the usual oxidation of chromogens. The comparatively small catalase increase might be thought of as a safety reaction destroying the peroxide in excess of the peroxide needs. The reaction may be represented as follows:

8. Sucrose + invertase  $\rightarrow$  glucose + fructose + peroxide group.

9. Hexose + anaerobic enzymes  $\rightarrow$  fermentation products.

10. Fermentation products indicated as RH<sub>2</sub>

![](_page_55_Figure_6.jpeg)

An examination of the curves for respiration, and enzymatic activity in the present work shows interesting relationships. The increase in respiration is paralleled by peroxidase and diastase. Catalase also increases but not conspicuously, while oxygenase actually decreases.

The divergence of the respiration and oxygenase curves shows clearly that any such mechanism as the oxygenase-cate-

chol system does not account for the increased respirational activities in this case.

The divergence of the peroxidase and oxygenase curves shows that the oxygenase-catechol-peroxidase system is not functioning.

The close correlation between the curves for respiration, invertase, and peroxidase indicates the possibility of the invertase-peroxidase series of reactions, and that this may be the mechanism for the increased respiration of the tomato plants grown in high potassium iodide solution. It seems probable that plants, such as horse-radish (*Cochlearia Armoracia*), which contain peroxidase but no oxygenase, might also depend on the invertase-peroxidase association. Certainly the possession of a strong peroxidase activity would be useless to a plant unless peroxides were available, either from such a reaction as suggested above for invertase or arising spontaneously by a non-enzymatic reaction.

## V. Conclusions

1. Potassium iodide, in the concentrations used, exerted only a depressing influence upon growth.

2. Toxic effects consisted of a loss of green color and a progressive dropping of the lower leaves. No definite spotting of the leaves was observed.

3. No significant change in the acidity of the expressed sap was observed. The pH remained very close to 5.9. This prohibited the possibility of increased enzymatic activity being

due to a more favorable pH of the sap.

4. Respiration, peroxidase, and invertase were decreased by a low concentration of potassium iodide (1 p.p.m.), but

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greatly increased at the higher concentration. This increase does not parallel the degree of iodine injury. The increase of catalase at the higher concentrations was much less than that of respiration, peroxidase, or invertase.

5. Oxygenase activity is progressively lowered in the plants growing in higher potassium iodide concentrations, thereby differing from the other oxidizing enzymes studied. This is

important evidence that the oxygenase-catechol system is not intimately concerned with the respiratory activity of the tomato plant.

6. The activity of peptase is not affected in the plants growing in different potassium iodide concentrations.

7. The stimulating effects observed on respiration and oxidizing activities of the injured plants are not similar to those induced by disease injury reported for other plants, since disease injury is known to increase also oxygenase.

8. A possible relationship of invertase to peroxidase and the effect of this relationship on respiration have been suggested, which may account for the increased respiration rate of the plant growing in the higher concentration of potassium iodide. This mechanism involves the formation of a transitory peroxide group during the formation of hexose from sucrose by the action of invertase. This peroxide may then be activated by peroxidase which oxidizes the hydrogen acceptor.

## VI. ACKNOWLEDGMENTS

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## EXPLANATION OF PLATE

PLATE 17

The respiration chambers (see Wynd, '32).

![](_page_65_Picture_4.jpeg)